Laboratory Update:

M.tuberculosis (MTB) Characterization, Nucleic Acid Amplification Test (NAAT), & Drug Susceptibility Testing (DST)

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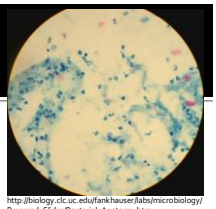
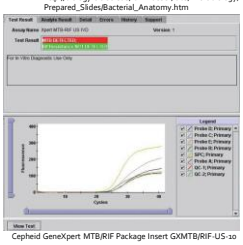
MTB Identification & Characterization



http://biology.clc.uc.edu/fankhauser/labs/microbiology/Prepared_Slides/Bacterial_Anatomy.htm

- Phenotypic Characterization
  - Microscopy
  - Morphology & Biochemical
  - Drug Susceptibility Testing (DST)
- Genotypic Characterization
  - DNA Fingerprinting
    - Spoligotyping
    - RFLP
    - VNTR analysis
  - Probe hybridization
    - Accu Probe
  - Nucleic Acid Amplification Test (NAAT)
    - GenProbe MTD, Cepheid GeneXpert
    - 16 S sequencing



Cepheid GeneXpert MTB/RIF Package Insert GXMTB/RIF-US-10

Mycobacteriology Testing: Annual Workload

2012

- Primary Isolation
  - 2963 patient samples
  - 807 individual patients
  - 55 individual patients positive for MTBC
- Reference Culture Identification
  - 616 patients
  - 85 individual patients positive for MTBC
- NAAT Testing (MTD)
  - 74 patients had the GenProbe MTD test performed
  - 30 patients had the MTD detect *M. tuberculosis* DNA

2013 (6 months)

- Primary Isolation
  - 1455 patient samples
  - 387 individual patients
  - 27 individual positive for MTBC
- Reference Culture Identification
  - 371 patients
  - 67 individual patients positive for MTBC
- NAAT Testing (MTD)
  - 26patients had the GenProbe MTD test performed
  - 5 patients had the MTD detect *M. tuberculosis* DNA

2012 Mycobacteriology Testing: Drug Susceptibility

2012

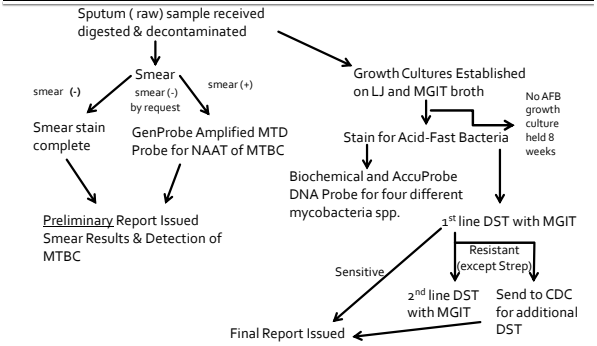
- First Line
  - 157 1st line DST on all initial *M. tuberculosis isolates*
- Second Line
  - 40 2nd line DST on all initial *M. tuberculosis isolates*

2013 (6 months)

- First Line
  - 54 1st line DST on all initial *M. tuberculosis isolates*
- Second Line
  - 12 2nd line DST on all initial *M. tuberculosis isolates*

DST for other *Mycobacteria* spp. available through National Jewish Hospital upon request.

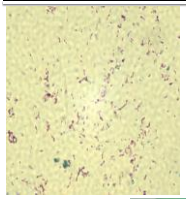
DCLS Current Workflow

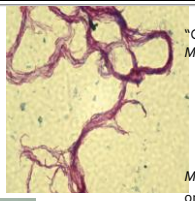


Continuing with the MGIT 960: Primary Isolation, 1st & 2nd Line DST

- Continuous incubation at 37°C & monitoring for fluorescence – based on O_2 concentration
- Growth of any organism is detected
  - Mycobacteria, yeast, other bacteria
- Smears prepared from broth
  - Growth determined to be acid-fast

Growth on LJ Slants & Acid Fast Staining

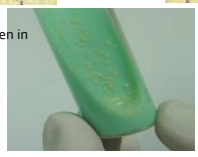




"Cording" typical of *M. tuberculosis*

M.tuberculosis on LJ slants

Pleomorphism and branching often seen in *M. avium* complex.



M.avium on LJ slants

Drug Susceptibility Testing: Performed on MGIT 960

- First line drug testing
  - Isoniazid (INH), Rifampin, Ethambutol, Streptomycin, Pyrazinamide (PZA)
- Results available within 7-12 days after speciation
- Resistant strains - results phoned to submitter
- Second line drug testing
  - Ethionamide, Capreomycin, Ofloxacin, INH at a higher concentration
  - Sent to CDC for additional drug susceptibility testing

GEN-PROBE Amplified MTD Test: DNA Probe for MTBC

- FDA approved the GenProbe Amplified *M. tuberculosis* Direct Test for AFB smear (+) respiratory specimens in 1995 and for smear (-) respiratory specimens in 1999
- Amplified molecular assay detects *M. tuberculosis* directly from sputum samples in less than 3.5 hours

GEN-PROBE Amplified MTD Test: DNA Probe for MTBC

Approved for: Respiratory Specimens

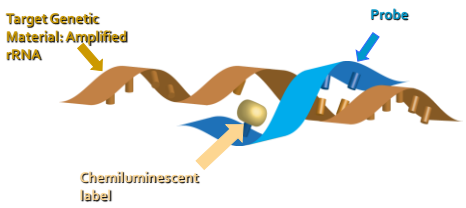
- Testing smear (+) and (-) specimens (NOTE: Smear (-) specimens NOT routinely tested at DCLS)
- **Testing patients who have taken TB medications for LESS than 7 days**
- Patients with high clinical suspicion of TB

NOT Approved for:

- Specimens from patients receiving TB medications in the past 12 months
  - NOT a test of cure
- Testing children or patients unable to produce sputum

GEN-PROBE Amplified MTD Test: DNA Probe for MTBC

- Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA



Cepheid GeneXpert MTB /RIF Implementation

- Performed on sputum samples (raw or processed) in ~2h with little hands on time
- In combination with Smear results, detects within 1-2 days of receiving sample:
  - Smear +/-
  - MTBC present
  - Mutation indicative of RIF resistance
- GenProbe Amplified MTD will serve as a backup method

Methodology Comparison

	Gen-Probe Amplified MTD Test†	Hains Genotype MTBDR plus Test‡	Cepheid GeneXpert MTB/RIF*
FDA Approval?	YES	YES	YES
Detection of:	MTBC only	MTBC & Resistance	MTBC & Resistance
Method:	Transcription-Mediated Amplification of rRNA	PCR + DNA-Strip hybridization	Nested real-time PCR
Sample type:	Sputum Sediment and bronchial specimens	Pulmonary specimens & isolates	Raw Sputum or Sputum Sediment
Time-to-Result	2.5-3.5h	5h	2h
Labor Intensive	YES	YES	NO
Detection of MTBC in Smear (+) Sensitivity/Specificity	87.5%/100%	100%/NA	99.7%/98.5%
Detection of MTBC in Smear (-) Sensitivity/Specificity	64.0%/100%	80.3%/98.4%	76.1%/98.8%
RIF Sensitivity/Specificity vs. Convention DST	NA	NA/100% (low sample volume)	94.7%/99.0%
INH Sensitivity/Specificity vs. Convention DST	NA	NA/100% (low sample volume)	NA

†GenProbe Amplified MTB users guide IN0014 Rev. P

‡Hains GenoType MTBDRplus users guide v2.0 IFU-304A-02. 02/2012

*GeneXpert MTB/RIF users guide 301-1404 Rev. A July 2013

What is the GeneXpert MTB/RIF Assay?

- Nucleic acid amplification test (NAAT)
  - Detects both MTBC and RIF resistance
- Test takes 2h from start to finish

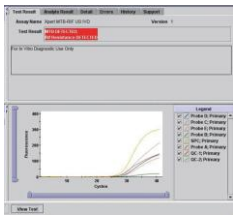
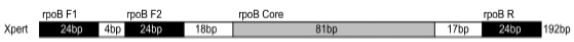


Figure 6. An Example of a MTB DETECTED; Rif Resistance DETECTED Result

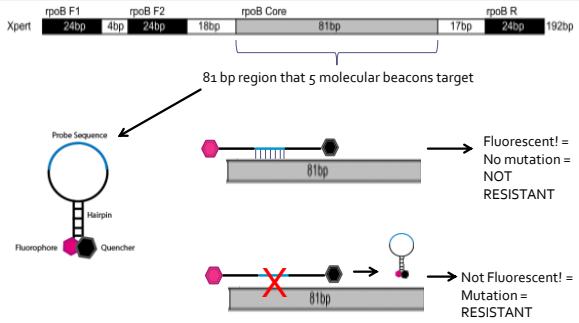
Principle of the GeneXpert Test

- Uses a hemi-nested PCR to amplify the *rpoB* gene in MTB
- Simultaneously probes the PCR amplicon for antibiotic resistance markers.

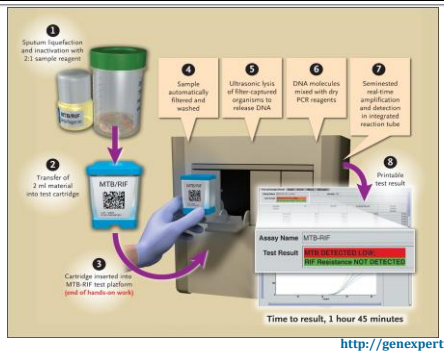


Blakemore, R., et al. 2010. JCM. 48:7. 2495-2501

Principle of the GeneXpert Test part II



GeneXpert MTB/RIF Process



http://genexpertinfinity.com/

Sample Requirements

Required Specimen Volume*

Specimen Type	Minimum Volume for One Test	Minimum Total Volume for Test and Retest – See Section 11.2, Retest Procedure
Sputum sediment	0.5 mL	1 mL
Raw sputum	1 mL	2 mL

- Samples should be stored & transported at 2-8˚C
- Test is only approved for induced or expectorated sputa
- **Samples from patients on antituberculosis drugs for >3 days are NOT acceptable.**

*GeneXpert MTB/RIF users guide 301-1404 Rev. A July 2013

Results & Potential Reporting

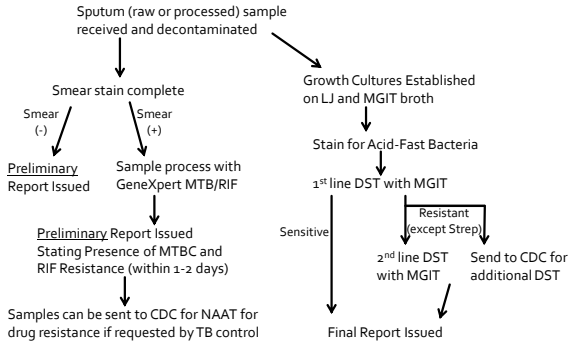
Xpert® MTB/RIF Readout	Interpretation	Report* (Suggested Minimal Language)
MTB DETECTED; RIF Resistance DETECTED	A mutation in the *rpoB* gene has been detected. A full first and second line drug panel should be conducted.	*rpoB* mutation detected; likely rifampin resistance; Confirmatory testing in progress OR isolate has been forwarded to a reference laboratory for confirmatory testing.
MTB DETECTED; RIF Resistance NOT DETECTED	A mutation in the *rpoB* gene has not been detected.	No *rpoB* mutation detected; likely rifampin susceptible.
MTB DETECTED; RIF Resistance INDETERMINATE	A mutation in the *rpoB* gene could not be determined due to insufficient signal detection.	Insufficient MTB in the sample to allow determination of *rpoB* mutation result.
MTB NOT DETECTED	**The conserved sequences up- and downstream of the 81bp region were not detected.**	**MTBC not detected; Confirmatory testing in progress.**

APHL Fact Sheet: Sept. 2013

Limitations

- Test has not been evaluated for pediatric patients
- Performance of assay relative to HIV infection status is not known
- Positive MTBC result ≠ viable organisms
- Test does not differentiate between species of MTBC
- Limit of Detection

Proposed DCLS 2014 Workflow



GeneXpert MTB/RIF Initial Presumptive Diagnostic Test

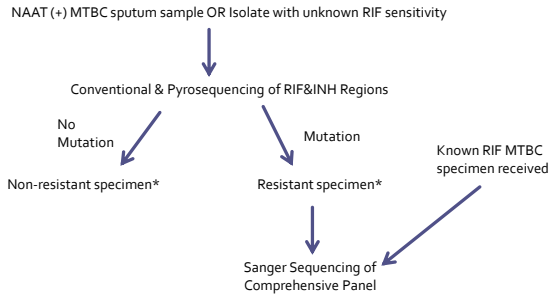
- False positive/negative results are possible
  - Therefore, this test provides preliminary results only
- Smear & MTB/RIF results will be sent as a preliminary report
- Culture and growth based DST will still be conducted
  - MGIT 960 used to confirm
- Final report will be a culmination of all preliminary reports
  - Molecular Detection of Drug Resistance (MDDR) from CDC will be included as an attachment.

CDC MDDR Assay

- DNA Sequencing to Detect 1st and 2nd line Drug Resistance (2-3 day TAT).

Gene Loci	Associated Antibiotic Resistance
rpoB (81bp region)	Rifampicin
inhA (promoter region)	Isoniazid
katG	Isoniazid
embB	Ethambutol
pncA	Pyrazinamide
gyrA	fluoroquinolones
rrs	Kanamycin, Amikacin, Capreomycin
tlyA	Capreomycin
eis (promoter region)	Kanamycin

CDC MDDR Procedure



*Based on NAAT. Confirmation by growth still necessary

Limitations of ALL Molecular Testing

- Gaps in Knowledge
  - What/when do mutations REALLY confer resistance
  - Not all mechanisms of resistance are known
- Limits of Detection
- Absence of mutation does not necessarily mean susceptible
  - Mutation does not necessarily mean resistant
- Conventional detection & DST still required

Conclusions

- DCLS will implement the Cepheid GeneXpert technology in the 1st quarter 2014
- Culture and growth based DST are still the gold standard
- CDC MDDR service can help with early detection of drug resistance. Requests for testing must go through TB Control in collaboration with DCLS
- DCLS and TB Control are available to assist with patient consultations and interpretation of results

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